

Air pollutants enhance rhinoconjunctivitis symptoms in pollen-allergic individuals

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Background: Little is known about the relation of airborne pollen allergens to nasal and ocular symptoms in combination with air pollutants.

Objective: The hypothesis was that air pollutants exacerbate allergic symptoms of the nose and eyes during the pollen season. In addition, the use of allergen measurements instead of pollen counts should be tested.

Methods: Fifteen pollen-allergic, nonsmoking subjects with weak reactivity of the airways recorded rhinoconjunctival symptoms and medication every morning and evening throughout the pollen season. Symptoms were compared with air pollutants (nitrogen oxide [NOx], particulate matter smaller than 10 μm , and ozone) and birch and grass pollen counts or, alternatively, to airborne birch and grass allergens determined using ELISA-techniques. A multiple linear regression model was used which controlled for autocorrelation of the residuals of the time series (Cochrane-Orcutt approach). This model was applied to each subject individually, followed by calculations of summary scores for the group.

Results: Air pollution levels were moderate, often meeting air quality standards. Effect estimates (increase of score with 10-fold increase of concentration) were NOx = 1.06, $P < 0.01$; ozone = 1.59, $P < 0.01$; and pollen = 0.48, $P < 0.001$. Using allergen concentrations instead of pollen counts resulted in similar effect estimates. Using particulate matter smaller than 10 μm instead of NOx gave comparable but less consistent results.

Conclusions: Symptoms were related to moderate levels of pollutants, suggesting that rhinoconjunctival tissue is very sensitive to irritant stimuli during an ongoing allergic inflammation, and that susceptibility toward allergens might be increased in areas with increased levels of air pollutants. Allergen measurements seem equally usable as pollen counts to investigate rhinoconjunctivitis.

Ann Allergy Asthma Immunol 2001;87:311-318.

INTRODUCTION

Allergic diseases are considered very important, environmentally related diseases today. In Switzerland, the prevalence of self-reported hay fever increased in the last century from 0.8% in 1926 to 14.2% in 1991.¹ This might

be related to decreased contact with infectious agents,² changed living conditions,³ and air pollutants.⁴ Although the influence of air pollutants on the prevalence of atopy is still controversial,^{5,6} they seem to play a role in short-term allergic reactions. In epidemiologic studies on allergic asthmatic patients, effects of air pollutants were frequently reported.^{7,8} Clinical studies showed changes in airways responsiveness and inflammatory markers after controlled exposure to ozone (O_3) or nitrogen dioxide (NO_2).^{9,10} O_3 , NO_2 , and SO_2 were also found to decrease the allergen dose necessary to provoke a defined decrease in lung function.^{11,12} However, most studies focused on lung function and asthmatic symptoms

and only a few examined nasal response and rhinitis.¹³⁻¹⁵

Pollen counts are often used in epidemiologic surveys but also clinically for differential diagnosis and timing of immunotherapy. In recent years, immunologic methods to directly measure allergens on airborne particles were developed.¹⁶⁻¹⁸ However, the relationship between these allergen measurements and allergic effects have been investigated so far in few studies.^{19,20} A close correlation between allergen concentrations and symptom scores was reported¹⁹; but the frequency of respiratory hospital admissions was only weakly correlated to allergen concentrations.²⁰

The aim of this study was to investigate the influence of air pollutants on allergic symptoms and to compare pollen counts to allergen measurements. First, short-term effects of pollutants and pollen on allergic symptoms were analyzed using time-series regression methods. Then, the same analysis was performed using birch and grass allergen concentrations in place of pollen counts.

METHODS

Effect evaluation

Fifteen pollen-sensitized allergic patients from the Allergy Unit of the Department of Dermatology, University Hospital in Zurich, recorded symptoms, medication, and time spent outdoors every day during spring and summer of 1998 in the AM after rising and in the PM before going to bed.

Selection criteria for subjects were to live and work in nonsmoking environments within central Zurich, to be allergic to birch or grass pollen but not to indoor allergens such as mites, house dust, or fungi, and not allergic to

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Received for publication March 31, 2001.

Accepted for publication in revised form July 6, 2001.

pets with which they are in contact. After written and oral consent was obtained, subjects were interviewed by an allergologist and underwent skin prick testing with aeroallergens (Solu-prick, ALK, Hørsholm, Denmark) following European Academy of Allergology and Clinical Immunology guidelines.²¹ Subjects were defined to be allergic against a pollen species if they 1) showed prick-reactivity (diameter of wheal) of 3 mm or larger against the investigated pollen and 2) reported allergic symptoms during the corresponding pollen season. Before and during pollen season, a methacholine provocation test was performed with measurement of forced expiratory volume in 1 second (FEV₁) upon inhalation of increasing concentrations of nebulized methacholine up to a cumulative dose of 2,000 μ g.

Subjects filled out a symptoms diary and rated the strength of symptoms by answering the question, "How severe were your symptoms last night/today?" Definition of symptoms of rhinitis and conjunctivitis were those used in the ISAAC-guidelines.²² The rating was made on a scale from 0 to 10 with 10 being the highest ever-experienced symptoms. For statistical analysis, a symptom score rhinoconjunctivitis was formed by summing the ratings for rhinitis (running/plugged up nose) and conjunctivitis (itching/watering eyes) resulting in a total maximal score of 20.

Subjects were asked not to record symptoms if they stayed the whole day outside the city of Zurich and not to add symptoms later, when recording was forgotten. Every 4 weeks, subjects were contacted and asked to continue with the recording of symptoms until the end of pollen season.

Exposure evaluation

Air pollutants were measured at a stationary site representative for central Zurich by the City Department of Health and Environment using standard techniques according to federal regulations.²³ Nitrogen oxides (NOx) and O₃ were measured continuously and recorded as 30-minute mean values. Particulate matter smaller than 10

μ m (PM10) was sampled for 24 hours from midnight to midnight. From the NOx and O₃ values, 24-hour means of pollutants were calculated for each individual recording time.

Stationary pollen measurements were obtained from the Swiss National Weather Service (SMI-Swiss Meteo). Data were provided as 24-hour means from 8:00 AM to 8:00 AM of the next day. Sampling was performed on top of the SMI-Headquarters in Zurich with a Burkard pollen sampler (Rickmansworth, Hertfordshire, England).²⁴

Particle sampling and allergen analysis were performed as described elsewhere.^{18,25} In brief, particles were collected in central Zurich separated by size daily for 24 hours starting at 7:00 AM with a high-volume cascade impactor (Digitel, Volketswil, Switzerland).²⁵ Particles were analyzed on the content of the major allergens of birch (Bet v 1) and grass (Phl p 5) with sandwich-ELISA-techniques based on monoclonal antibodies.¹⁸

Model and Statistical Analysis

For each subject, the relationship between effect and exposure was examined individually. A model named "Pollen" investigated the combined short-term (last 24 hours) effects of exposure to NOx, O₃, and pollen. Separate analyses were carried out for symptoms in the morning, in the evening, and with all values. To the latter, a term AM/PM was added to account for systematic differences between morning and evening. The term was 0 in the morning and 1 in the evening. Exposure data were log-transformed before calculating the models. Pollen counts were shifted by one unit in advance because 0-values would otherwise have been lost in the transformation. Analysis was done separately for the birch and grass pollen seasons for subjects reacting to both pollen. The model "Allergen" was identical but used airborne allergen concentrations rather than pollen counts.

The residuals of the fitted model showed autocorrelation. Therefore, an approach following Cochrane and Orcutt²⁶ was used to fit linear regression

models with autocorrelated errors. First, the likelihood of the model was maximized using ordinary least-squares. Then, an autoregressive process was fitted to the residuals of the least-square fit, followed by a transformation of the model to remove autocorrelation of the errors (Cochrane-Orcutt transformation). The transformed model was then fitted again using least-squares. If data were missing, the likelihood of the model was fitted using the Kalman filter applied to a state space representation of the likelihood. For summary statistics over all subjects, means, standard errors, and *P* values were calculated from the effect estimates and standard errors of the individual regression models. In detail:

A single effect b_j was assumed to be distributed as $N(\beta_j, \text{var}(b_j))$, where β_j is the coefficient of the *j*-th variable, b_j is its estimate and $\text{var}(b_j)$ is the square of the standard error of b_j . Hypothesis: all effects β_j are 0. Test statistic is $\bar{b}_j = 1/n \sum b_j$ and $\text{var}(\bar{b}_j) = 1/n^2 \sum (se(b_j))^2$, test with standardized test statistic $\bar{b}_j/(1/n \cdot \sqrt{\sum (se(b_j))^2})$ which is approximately distributed as $N(0,1)$. Calculations were performed using SPlus 4.5 on Windows NT.²⁷

RESULTS

Subjects

Nine men and six women, aged between 22 and 39 years old (median 27) recorded their symptoms twice a day. Subjects were nonsmokers living in nonsmoking households. Three subjects were several times exposed to tobacco smoke at the workplace during breaks, but always for <1 hour a day. Two subjects cooked with gas, all others electrically. One subject had many missing values. Two recorded mostly zero symptoms; therefore, they had to be defined as nonsymptomatic. Consequently, only 12 subjects were analyzed. Table 1 shows a summary description of the subjects. Six of the studied subjects were allergic to grass, 3 to birch, and 3 to both pollen species, resulting in 15 analyses. Methacholine reactivity was small in all subjects. At the maximal cumulative dose of 2,000 μ g, FEV₁ was decreased by 10% (me-

Table 1. Characterization of the 12 Subjects Whose Data Were Analyzed

Subject	Gender	Age (y)	Medication	Methacholine reactivity
G1	m	24	no	3%
B1/G2	f	30	yes	8%–32%*
B2	f	29	yes	19%
G3	m	39	no	1%
G4	m	22	no	13%
G5	m	29	yes	21%
B3	f	26	no	5%
B4	m	23	no	24%
B5/G6	m	28	no	16%
B6/G7	f	24	no	1%
G8	m	27	yes	12%
G9	f	35	yes	10%

* 32% during an ongoing cold, 8% at repetition.

Subject code reflects birch (B) and grass (G) allergy. Medication indicates consumption of allergy-related drugs during the study period. Methacholine reactivity shows the decrease of FEV₁ at the maximal cumulated dose of 2,000 µg methacholine.

dian, range 0 to 24%) during pollen season. Only two subjects showed reactivity >20% and only at the maximal dose. A third subject showed a 32% decrease during an ongoing cold but only 8% during a repetition. Five of the 12 analyzed subjects regularly used medication however, with almost no variation throughout the season. Several subjects recorded a common cold for some days during the study period with symptoms different from the usual allergic symptoms. These days were excluded from the analysis.

Exposure

Air pollutants, birch and grass pollen, and their allergens on particles were measured from March 1st to August 8th, 1998. Figure 1 shows the time course of daily means of NO_x, O₃, and birch and grass pollen. Pollen seasons of birch and grass are clearly recognizable and well distinguishable, whereas pollutants show large variation but no seasonality and no obvious differences in the level of pollutants between birch and grass pollen season. Time courses of birch and grass allergens follow those of the pollen counts closely (data not shown¹⁸).

Table 2 shows the summary statistics of 24-hour means for air pollutants. Air pollution levels were moder-

ately with only 1 day exceeding the national threshold level of NO₂ (24-hour mean >80 µg/m³), 35 days exceeding the O₃ threshold of 120 µg/m³ level for the 1-hour mean and 3 days exceeding the daily threshold of PM10 of 50 µg/m³. Allergens were found mainly on particles larger than 10 µm. For PM10, allergen concentrations were mostly close to or below detection limit. Therefore, only the allergen load of particles larger than 10 µm was used

for statistical analysis. Correlation between exposure variables (NO_x, O₃, pollen, or allergen) entering into the same model was weak. Pollen counts were strongly correlated with the corresponding allergen concentrations (Table 2).

Rhinoconjunctival Symptoms

Rhinoconjunctival symptoms were recorded almost exclusively during the pollen season, but in some cases several days earlier. The pattern of symptoms resembled in most cases the pattern of pollen counts and allergen load of particles. The average symptoms score over all analyzed subjects throughout the season was 4.4 points on a scale of 0 to 20. Many subjects reported that symptoms were less than in other years and most subjects never reported rhinoconjunctival symptoms >10.

The influence of NO_x, O₃, and pollen or their allergens, respectively, was investigated in two models called Pollen and Allergen. At the level of individuals, effect estimates were mostly positive as shown in Figure 2 and reached significance for some subjects.

Summary statistics over all subjects are shown in Table 3. All mean effect

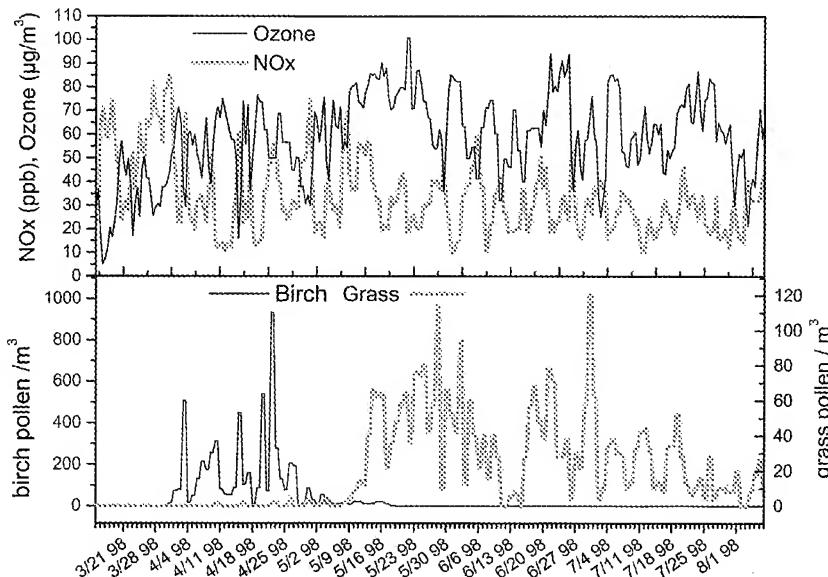


Figure 1. Time course of air pollutants and pollen in spring and summer, 1998, in Zurich.

Table 2. Summary of 24-hour Means of Exposure Variables from March 1 to August 8, 1998: Median, Range (Minimum to Maximum), Standard Deviation (SD), and Correlation Coefficients between the Log-Transformed Exposure Variables

	Exposure data			Correlation coefficients between exposure variables (log-transformed data)					
	Median	Range	SD	NOx	O ₃	Birch pollen	Birch allergen	Grass pollen	Grass allergen
Nitrogen oxides (NOx, ppb)	31.7	6.9-87.6	15.5	1	-0.53	0.04	0.15	-0.23	-0.24
Ozone (O ₃ , $\mu\text{g}/\text{m}^3$)	57.9	3.9-101.0	18.65	-0.53	1	0.04	-0.04	0.51	0.44
Birch pollen (counts/ m^3)	10	0-932	155	0.04	0.04	1	0.76	—	—
Birch allergen (extracted ng/mL)	0	0-183	27	0.15	-0.04	0.76	1	—	—
Grass pollen (counts/ m^3)	28	0-120	26	-0.23	0.51	—	—	1	0.89
Grass allergen (extracted ng/mL)	6	0-33	7.4	-0.24	0.44	—	—	0.89	1
Particulate matter (PM10, $\mu\text{g}/\text{m}^3$)	23.1	8.9-54.4	10.7	0.66	-0.17	-0.15	0.00	-0.03	0.00

estimates of NOx, O₃, and pollen or allergens, respectively were significantly positive. Effect estimates are the slopes to logarithmic exposure units in a model that controlled for autocorrelation, ie, they indicate the increase of symptom scores with a 10-fold increase of the corresponding exposure variables during the last 24 hours.

Larger values in the evening compared with the morning were seen for intercept, O₃ and pollen or allergen, respectively. To control for time-series effects, a first order autoregressive process had to be fitted to the morning and the evening models and a second order process to the combined models. The mean autoregression coefficients were as follows: morning, 0.23 and 0.22 for Allergen and Pollen, respectively; evening, 0.35 and 0.31; and combined 0.25 (lag 1)/0.18 (lag 2) and 0.25/0.17.

Between the two models Allergen and Pollen, mean effect estimates were similar. For a further comparison, Pearson correlations between the individual coefficients of the two models were calculated (Table 4). For all explanatory variables the effect estimates of the two models correlated well and significantly with one another.

In a subsequent run, the rhinoconjunctivitis models were calculated using concentrations of PM10 instead of NOx (Table 3). For the AM exposure, PM10 values of the previous day were taken, and for the PM, the values of the current day. At the level of individuals, only in a few cases were significant effect estimates found. The results of

the evening models were similar to the models with NOx. The other models gave less consistent findings (Table 3).

DISCUSSION

In this study, we demonstrate the influence of air pollutants and birch and grass pollen or allergens, respectively, on rhinoconjunctival symptoms. Until now, little was known about this relationship because studies dealt mostly with the respiratory tract of allergic, asthmatic patients.^{7,8}

Summarizing effect estimates (Table 3) shows that, during the pollen season, allergic symptoms are significantly associated to concentrations of air pollutants of the last 24 hours. The order of autocorrelation indicates that the last 48 hours influence the symptoms score significantly. Coefficients for NOx and O₃ were larger than coefficients for pollen or allergen. This suggests that even low concentrations of pollutants, which occur frequently in Zurich, have an important influence on an ongoing allergic inflammation represented by the symptoms score. Note that an increase of 1 symptom point reflects an increase of approximately 25% of the average symptoms score recorded during the pollen season. However, pollen or allergens remain very important also for short-term reactions because they vary over several orders of magnitude, as seen in Table 2 and in Figure 1, whereas air pollutants vary much less.

Clinical human studies report changes in inflammatory markers and lung function after exposure to NO

(nitric oxide) and NO₂,^{10,28} as well as SO₂ (sulfur dioxide) combined with NO₂.¹² PM10 affects lung function and morbidity,²⁹ and in vitro studies associate PM10 with inflammation.³⁰ O₃ is known to irritate nose and airways after exposure to high concentrations in healthy as well as allergic subjects.^{31,32} In epidemiologic studies, O₃ effects on lung function were reported at concentrations as low as 150 $\mu\text{g}/\text{m}^3$.⁸

During our study period concentrations of every single pollutant were lower than effect concentrations found in the aforementioned studies. This suggests that the rhinoconjunctival epithelium is very sensitive to irritant stimuli during an ongoing allergic inflammation. However, in the urban environment, daily NOx correlates well with NO₂ and NO ($r > 0.9$ in Zurich during study period), SO₂ ($r = 0.75$) and PM10 ($r = 0.66$). Therefore, effect estimates of NOx and O₃ may as well reflect the influence of the mixture of several pollutants. The contribution of indoor air pollutants is probably small as the subjects were nonallergic to indoor allergens, nonsmokers, and lived in mostly nonsmoking environments without gas cooking.

No significant difference was seen between the two models Pollen and Allergen. The coefficients for allergen correlate well with the coefficients for pollen (Table 4), which is explained by the good correlation between the two exposure variables. For rhinoconjunctivitis symptoms, allergen measurements seem to give similar information as pollen counting.

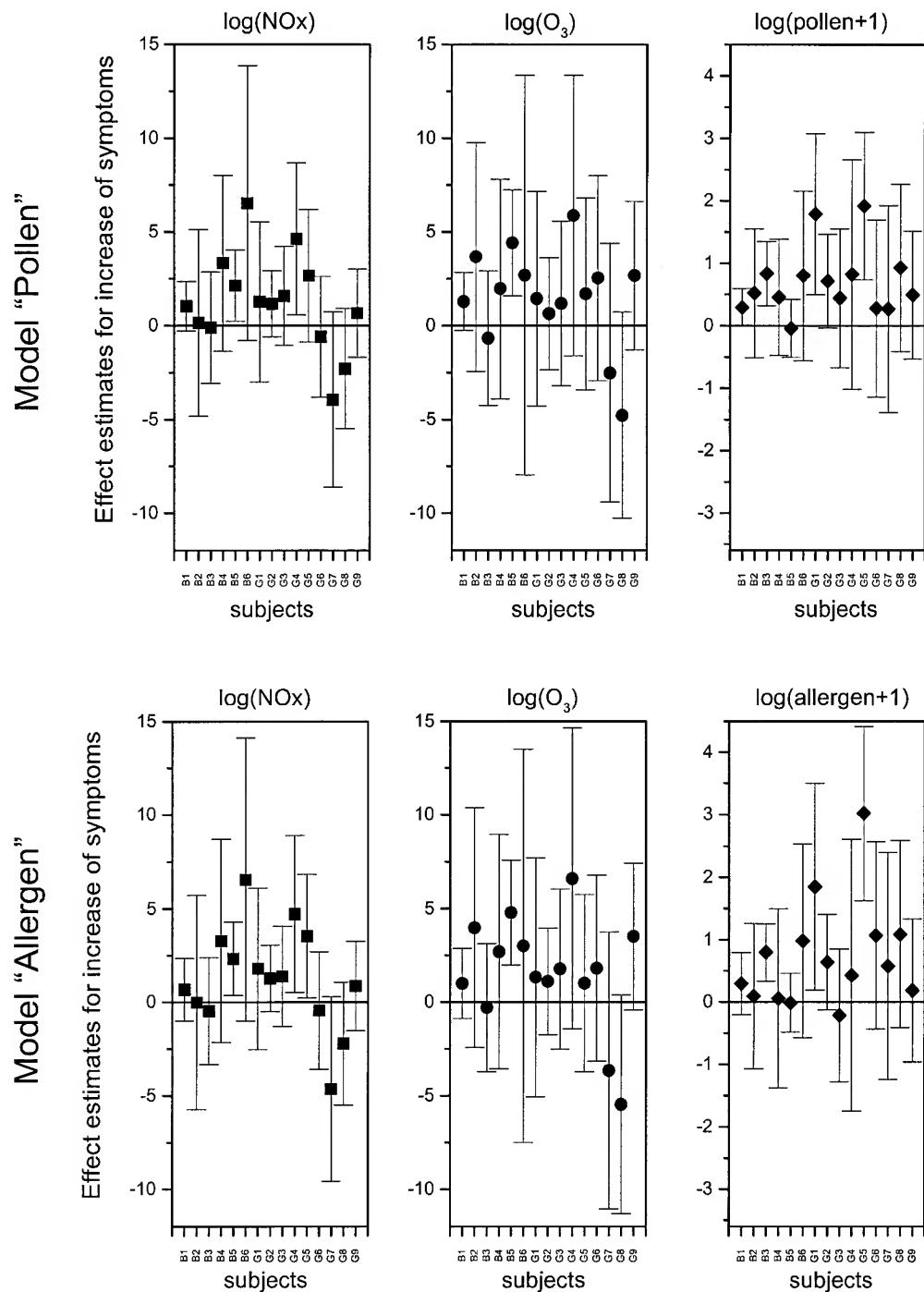


Figure 2. Individual effect estimates with 95% confidence intervals for the exposure variables of the models "Allergen" and "Pollen" in the submodel combining morning and evening symptoms. Effect estimates indicate the increase of the symptom score at a 10-fold increase of the exposure variable.

Exploratory analyses showed similar effect estimates for PM10 as for NOx in the evening models (Table 3). However, the morning and the combined models revealed vanishing effect

estimates for O₃, and small and not consistently significant effect estimates for PM10. We attribute the differences between the models to uncertainties in the estimation of the PM10

exposure, and to the fact that NOx represented the primary pollutants better than PM10 in our study.

In all models on rhinoconjunctivitis, the intercept was negative (Table 3).

Table 3. Effect Estimates for Rhinoconjunctival Symptoms: Summary over All Subjects

	Main models					
	Model "Allergen"			Model "Pollen"		
	Morning	Evening	Combined	Morning	Evening	Combined
log(NO _x)	1.12*** (0.48, 1.75)	1.18* (0.08, 2.28)	1.06** (0.34, 1.78)	1.08*** (0.49, 1.68)	1.41** (0.40, 2.41)	1.06** (0.39, 1.73)
log(O ₃)	1.07* (0.15, 1.99)	3.15*** (1.59, 4.72)	1.59** (0.59, 2.59)	0.99* (0.09, 1.88)	3.00*** (1.60, 4.39)	1.47** (0.53, 2.42)
log(Allergen)/ log(Pollen)	0.25** (0.07, 0.43)	0.78*** (0.44, 1.12)	0.48*** (0.26, 0.70)	0.20* (0.03, 0.37)	0.71*** (0.45, 0.97)	0.46*** (0.27, 0.64)
AM/PM			1.06*** (0.88, 1.24)			0.95*** (0.78, 1.11)
Intercept	-2.52* (-4.72, -0.32)	-4.40* (-8.29, -0.50)	-2.96* (-5.44, -0.47)	-2.48* (-4.55, -0.41)	-5.08** (-8.56, -1.60)	-3.02* (-5.33, -0.71)

	Models calculated with PM10 instead of NO _x					
	Model "Allergen"			Model "Pollen"		
	Morning	Evening	Combined	Morning	Evening	Combined
log(PM10)	0.85* (0.12, 1.58)	1.64** (0.41, 2.87)	0.98* (0.1, 1.86)	0.78* (0.08, 1.47)	1.37* (0.18, 2.57)	0.71 (-0.20, 1.61)
log(O ₃)	0.26 (-0.53, 1.04)	2.49*** (1.20, 3.77)	0.88 (-0.06, 1.81)	0.21 (-0.57, 0.99)	2.26*** (1.03, 3.49)	0.69 (-0.30, 1.67)
log(Allergen)/ log(Pollen)	0.35*** (0.15, 0.56)	1.02*** (0.64, 1.40)	0.64*** (0.39, 0.89)	0.21* (0.03, 0.40)	0.90*** (0.58, 1.22)	0.56*** (0.32, 0.81)
AM/PM			1.08*** (0.88, 1.28)			1.10*** (0.90, 1.30)
Intercept	-0.24 (-1.82, 1.34)	-3.66* (-6.73, 0.59)	-1.05 (-3.11, 1.00)	-0.29 (-1.83, 1.24)	-3.57* (-6.56, -0.59)	-0.78 (-2.89, 1.32)

Means (95%-confidence intervals) were calculated from the individual effect estimates (see Figure 2). Estimates are the slopes to log-scaled exposure units, i.e. they indicate the increase of symptom scores at a ten-fold increase of the exposure variables. AM/PM is the estimate for the systematic difference between morning and evening. All mean effect estimates of both models are significantly different from zero. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

This suggests the existence of a threshold level below which pollen or their allergens do not provoke any symptoms. Such a threshold level is plausible and was previously reported for allergen provocation³³ as well as for combined effects of O₃ and allergens.¹⁴ The model suggests that the threshold is lowered by increasing air pollution.

In our combined model, the coefficient representing the difference between morning and evening (AM/PM, Table 3) indicates that symptoms were systematically larger in the evening. Circadian changes of hormones and inflammatory mediators normally lead to decreases in inflammation during the day.³⁴ We therefore attribute the increase to the exposure to pollutants and pollen, which might be increased during the day because of outdoor activities.

Summaries of effect estimates are significant for all variables of the rhinoconjunctivitis models, as shown in Table 3. This seems surprising for such a small number of subjects. However, we followed the subjects over several months during the entire pollen season. This has advantages for exposure evaluation and for consideration of inter-

personal variability: studies on exposure evaluation for air pollutants^{35,36} report that in longitudinal analysis, (ie, when following subjects over a certain time) the correlation between personal ("real") exposure and stationary measurements is better than in cross-sectional analysis (ie, when comparing different subjects at the same time).

Table 4. Correlations (Pearson *r*) between the Individual Effect Estimates for Rhinoconjunctivitis of the Models "Allergen" and "Pollen."

	Rhinoconjunctivitis		
	Morning	Evening	Combined
NOx	0.95	0.95	0.99
Ozone	0.80	0.96	0.99
Allergen/Pollen	0.69	0.70	0.86
AM/PM			1.00
Intercept	0.91	0.97	1.00

All correlations are significant (P < 0.005).

Air pollutants were measured at a location representative for central Zurich, which was the area our subjects spent most of their time. The confounding influence of indoor air pollutants was minimized by the selection criteria for the subjects (nonsmokers, smoke-free environment, no indoor allergies). In the absence of indoor sources, NO_2 indoor/outdoor relationships are strong.³⁷ For O_3 , however, indoor/outdoor ratios are smaller.³⁷ At the same time in the same area with a similar group of subjects, we conducted a study on the personal exposure to pollen.³⁸ Interestingly indoor pollen concentrations reached approximately 20% of outdoor levels. The results suggested that stationary measurements are usable for estimating personal pollen exposure throughout a pollen season, although correlation between pollen reports and personal pollen exposure shows large scattering because of differences in the individual activity patterns.³⁸

Because we followed subjects over a long time period, each diary could be analyzed individually, which avoids problems of interpersonal variability caused by differences in susceptibility, recording strategies, and time-activity patterns. However, the coefficients of the individual subjects were significant only occasionally, even for the effects of pollen. Two subjects (B5 and G3) had even slightly negative estimates for pollen or allergen, although the symptoms were restricted to the pollen seasons. This indicates the extent of difficulties in symptom recording and exposure evaluation. Most subjects had missing values in their diaries, thereby enhancing the uncertainty of the autocorrelation model.

Note that a quantitative interpretation of estimated effects is hampered by the attenuation effect; because the exposure of the individuals is presumably measured with a large margin of random error, the estimated effects will be smaller than the true effects.³⁹ In addition, because the data were collected in a single season and the measurements of air pollutants and aller-

gens were essentially the same for all subjects, the variability of the coefficients between seasons and regions could not be evaluated. (Similar restrictions apply to most studies of health effects.)

CONCLUSION

Rhinoconjunctival symptoms in pollen-allergic patients seem to be strongly influenced by air pollutants represented by NO_x and O_3 throughout the pollen season. Therefore, susceptibility toward allergens might be increased in areas with increased levels of air pollutants. Effect concentrations seem to be lower than those found in studies examining the airways of asthmatic patients. This is important for designing future studies using rhinoconjunctivitis symptoms for investigating allergic diseases. The comparison of allergen measurements to pollen counts showed that both techniques are equally usable for the investigation of rhinoconjunctival symptoms.

ACKNOWLEDGMENTS

We thank Dr. Bernhard Weber, Allergopharma Joachim Ganzer KG, Reinbeck, Germany for providing antibodies for the allergen detection; Dr. Alfred Meier, Department of Public Health and Environment (AGU) of the city of Zurich for providing pollutant data; Dr. Regula Gehrig and Dr. Thomas Frei, SMI-Swiss Meteo Zurich for the pollen data; and Dr. Lisa Pritscher from the Seminar for Statistics of ETH Zurich for her close cooperation with the statistical analysis.

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